REPORT

OF THE

BOMBAY BACTERIOLOGICAL LABORATORY

FOR THE YEAR 1913,

BY

MAJOR W. GLEN LISTON, C.I.E., M.D., D.P.H., I.M.S.,

Director, Bombay Bacteriological Laboratory.

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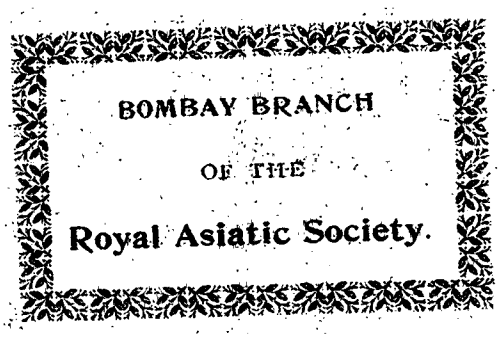

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REPORT OF THE BOMBAY BACTERIOLOGICAL LABORATORY FOR THE YEAR 1913.

By Major W. Glen Liston, C.I.E., M.D., D.P.H., I.M.S., Director, Bombay Bacteriological Laboratory.

STAFF.

Staff.

1. The post of the Director of the Laboratory was held by Major Wm. Glen Liston, C.I.E., M.D., D.P.H., I.M.S., throughout the year except during the months of June, July, August and September when Major T. H. Gloster, M.B., D.P.H., I.M.S., acted as Director.

The Assistants of the Director were :—

(*a*) Major T. H. Gloster, M.B., D.P.H., I.M.S., during the whole year except for the period when he acted for the Director.

(*b*) Captain W. D. H. Stevenson, M.B., I.M.S., Assistant to the Director, from January to September, proceeded on leave, 3rd October 1913.

(*c*) Captain J. Taylor, M.D., D.P.H., I.M.S., joined the Laboratory vice Captain W. D. H. Stevenson, I.M.S., on leave as Assistant to the Director from 3rd October 1913 in addition to his duties as Member, Plague Research Commission.

(*d*) Captain J. Morison, M.B., I.M.S., continued to work on special duty in Poona investigating the cause and prevalence of diarrhœa from January to the end of November, proceeding on leave from 1st December 1913.

ARRANGEMENT OF THE REPORT.

2. The report is divided into two parts setting forth under separate heads the work done :—

(*a*) as the Laboratory in which all the anti-plague vaccine used in India and its dependencies is made, and in which investigations connected with plague are carried out ; and

(*b*) as the Bombay Bacteriological Laboratory in which work connected with preventive medicine and pathological diagnosis for the whole of the Bombay Presidency is carried out.

PART I.

Work connected with Plague.

(*a*) Routine Work.

Output in doses of anti-plague vaccine for the past 5 years.

3. The table below gives comparative figures of output in doses of anti-plague vaccine for the past five years :—

1909.	1910.	1911.	1912.	1913.
593,164	625,690	1,211,170	727,377	795,637

It will be seen from this and the following statement that the output of the anti-plague vaccine has been greater as compared with that of the previous

year. This was due to the increase in the demand from countries outside India, especially the British East African Protectorates. The only places in India where there was an increase in the demand for the plague prophylactic were the Bombay Presidency and Burma.

Since the Laboratory was opened in 1896 up to the end of 1913 the total number of doses sent out has been 11,429,854.

The distribution of anti-plague vaccine.

The distribution of anti-plague vaccine in India and foreign countries during the last two years is shown below (figures represent number of doses) :—

Place or Province.	1912	1913.
Bombay Presidency and Sind	99,501	220,575
Madras Presidency	135,016	119,460
Bengal, Bihar and Assam	14,265	12,800
United Provinces of Agra and Oudh	176,355	126,192
The Punjab	66,865	46,800
North-west Frontier Province	1,400	1,170
Central Provinces and Berar	52,450	13,060
Burma	5,510	40,200
Native States in India	126,295	62,255
Countries outside India	49,420	152,625
Portuguese Territory in India	300	500
Total number of doses ...	727,377	795,637

The monthly despatch was as follows :—

Month.	1912.	1913.
January	123 885	46,315
February	99,107	61,760
March	56,747	56,896
April	34,477	52,010
May	8,836	8,185
June	9,610	8,475
July	15,631	20,140
August	42,503	131,775
September	113,965	124,015
October	73,630	151,165
November	51,286	93,116
December	97,700	41,785
Total number of doses ...	727,377	795,637

Statistics of inoculation.

Statistics of inoculation.

4. The usual Circular Letter was issued from the Laboratory with the object of collecting as far as possible reliable statistics on inoculation. Unfortunately the majority of replies received to this letter were incomplete in some or many respects, and only a selection of the more carefully compiled statistics is given below. Even these selected, however, cannot be regarded as statistically perfect but they serve to indicate the advantages of this method of combating the plague and show that the standard of the efficiency of the anti-plague vaccine prepared at the laboratory is well maintained.

The Bombay Presidency.

Bijápur District.

The following statistics were received through the Sanitary Commissioner and have been compiled by Temporary Assistant Surgeon D. R. Vaishnav working in the Bijápur District. These statistics are of interest and importance because a house-to-house inquiry was made after the close of the epidemic.

Amingad. Amingad is a small village which, according to the census of 1911, had a population of 4,339 persons. Rats began to die in this village in October 1912 and the first human case of plague was reported on 1st November 1912. The village was first visited by Assistant Surgeon Vaishnav on 24th November 1912 after a few cases of plague had occurred. The people were however unfavourable to inoculation so that only a few persons were inoculated up to the 3rd December. Some of these contracted plague but speedily recovered, so that when Assistant Surgeon Vaishnav again visited the village on the 16th December the people crowded to him for inoculation and within a few days more than fifteen hundred persons were inoculated. All these persons were not however living in the village, for some were in huts outside the village. A rough census of the population living in the village, taken at the time the inoculations were carried out, showed that of a population of 4,339 about 1,950 were living in the village and of this number about 1,000 were inoculated. A more careful house-to-house inquiry made at the close of the epidemic showed that throughout the epidemic 1,767 persons continued to live in the village ; 953 of these were inoculated, while 814 were uninoculated. Among the inoculated there were 10 attacks and 4 deaths from plague, while among 814 uninoculated there were 93 attacks and 81 deaths. There was in addition one death from other cause than plague among the inoculated, while four persons died from other causes than plague among the uninoculated. We have been able to compile more accurate statistics from the notes taken at the house-to-house investigation which was made at the close of the epidemic. In forty-eight households in which inoculated and uninoculated persons were living together throughout the epidemic plague cases occurred at some date subsequent to the inoculation of the last inoculated person in the household. In these forty-eight households 177 inoculated persons lived with 154 uninoculated persons. Among the 177 inoculated there were 8 attacks and 2 deaths while among the 154 uninoculated persons there were 57 attacks and 48 deaths from plague. If the inoculated in these infected households had suffered to the same extent from plague as the uninoculated they would have had 65 attacks and 55 deaths in place of 8 and 2 respectively, so that in this little village of four thousand inhabitants, it appears that at least fifty-three lives were saved by inoculation.

Rakhasgi. An epidemic of extraordinary severity broke out in the little village of Rakhasgi, population 772, on the 14th January 1913. During this short period 41 persons were attacked and 35 of these died. Inoculation began on the 24th January and by the 28th 308 persons were inoculated. Of the remaining population 247 uninoculated remained in the village with the inoculated persons above mentioned while the remainder sought refuge in camps outside the village. After the completion of inoculation, among the inhabitants living in the village throughout the epidemic, there were 28 attacks and 28 deaths among the uninoculated, while there were only 2 attacks and 2 deaths among the inoculated. One of the inoculated who died developed the disease on the same day as he was inoculated while the other contracted infection nine days after inoculation. In addition to the deaths from plague during the period of the epidemic, an uninoculated female died of phthisis and an uninoculated child aged four years died of broncho-pneumonia. From the notes compiled after the completion of the epidemic we found that plague appeared in twelve houses in which inoculated persons were living beside uninoculated after the date on which the last inoculated person had been inoculated. In these twelve houses there were 34 inoculated persons and 39 uninoculated and among these there were 17 deaths from plague among the uninoculated while there were only two among the inoculated. If the inoculated had suffered from plague to the same extent as the uninoculated they would have had fourteen deaths in place of two only so that we may estimate that in this village at least twelve lives were saved by inoculation.

Kamatgi. The village of Kamatgi, having a population of 4,957 persons, was attacked by plague on the 16th November 1912. The epidemic persisted till the 10th February 1913. Inoculation was commenced on the 5th December, the last inoculation being performed, on the 4th January. When inoculation was commenced it was found that many persons had left the village. A rough census taken at the time showed that only 807 persons out of a population of 4,957 remained in the village. Of the

807 persons 456 were inoculated but owing to the continued severity of the epidemic some of them left the village. A house-to-house inquiry at the close of the epidemic showed that only 292 inoculated and 392 uninoculated remained in the village throughout the epidemic, while there were 97 attacks and 97 deaths among the 392 uninoculated; there were only 4 attacks and 3 deaths among the 292 inoculated. The attacks among the inoculated occurred one four days, 2 five days and 1 forty-two days after inoculation.

Other villages in the Bijápur District.

Similar statistics have been compiled for other villages in the Bijápur District. The figures deal with separate households and do not include any cases of plague which occurred prior to the commencement of inoculation. Only those houses have been considered in which inoculated were living with uninoculated and in which plague appeared ten days after inoculation was completed. The Sanitary Commissioner for the Government of Bombay who supplied us with these statistics remarks that "these conditions naturally lessen the number of statistics but give the true protective value of inoculation, seeing that for inclusion in these statistics a household must have been exposed to infection after the operation of inoculation was performed" :—

Name of Village	Inoculat d.	Uninoculated.	Inoculated.		Uninoculated.	
			Cases.	Deaths.	Cases.	Deaths.
Ilkal	41	38	9	...	37	36
Herur	41	28	3	1	9	7
Gorubal	42	43	3	2	20	19

Tondihal.

The outbreak of plague in the small village of Tondihal is of interest. In this village the whole population of 153 persons were inoculated before plague began because a severe epidemic was raging in Gorubal and Ilkal which were distant a half mile and a mile respectively from Tondihal. Although rats died in the village from the 15th August to the 10th October in such numbers that in every house dead rats were found at some time or another, only four persons were attacked by plague and all recovered.

Sulebhadi.

A somewhat similar experience was recorded in the village of Sulebhadi which has a population of 3,392 persons, of whom 1,832 were inoculated. The people ascribed the mildness of the epidemic to inoculation which was especially practiced in the Kurub and Hatigar Peths, where a severe epizootic was prevalent. Only 14 attacks and 11 deaths were reported throughout the epidemic. Among other instances where the beneficial effect of inoculation was noticed the following is especially striking and noteworthy. In one household containing seven persons, three of whom were inoculated while four were uninoculated, three uninoculated contracted and died of plague while only one inoculated suffered from the disease but recovered.

Brew and age of vaccine.

It is perhaps worthy of mention that the anti-plague vaccine used in the Bijápur District had been brewed for six weeks and had been bottled for nine months. Assistant Surgeon Vaishnav who performed most of the inoculations states that he had neither heard of nor seen any ill-effects from inoculation. The material did not produce a severe re-action.

THE MADRAS PRESIDENCY.

Coimbatore.

An extensive inoculation campaign was carried out in Coimbatore town by Khan Sahib Gulam Hussain Sahib Bahadur, the Honorary Plague Officer, under the guidance of the district officers. Out of a population of a little over 55,000 nearly 46,000 were inoculated. Dealing with such a large number of persons it is very difficult to obtain absolutely accurate figures. The

Honorary Plague Officer in an explanatory note to the table printed below states that "the estimated population of the Coimbatore Municipality was 47,007 at the commencement of the epidemic in 1913. Deducting 17 deaths from plague which occurred prior to the commencement of the inoculation campaign, the estimated population on September 3rd, 1913, was taken as 46,990".

"The conditions which affected the weekly population during the course of the epidemic and which have to be taken into account are four, *viz.* :—

(1) Reduction by deaths.
(2) Reduction by compulsory evacuation.
(3) Increase by reoccupation.
(4) Increase by extension of municipal limits.

Each of these conditions have been taken into account in arriving at the weekly population in the accompanying table."

Statement showing attacks and deaths from plague among inoculated and uninoculated persons in Coimbatore Town from the commencement of the Inoculation campaign, 3rd September 1913, to the end of the epidemic, 4th April 1914.

The total population of the old town ... ... 47,007 } The total population after deducting the
Deaths prior to the commencement of inoculation ... 17 } deaths at the commencement of inoculation 46,990

Month.	Week.	Population at the commencement of the week.	Increased by		Decreased by		Total number of inoculated	Total number of uninoculated.	Inoculated.		Uninoculated.		Deducting the number of attacks and deaths from the inoculated within the incubation period of 7 days.	
			Reoccupation.	Extension of municipal limit.	Compulsory evacuation.	Deaths during weeks among inoculated and uninoculated.			A.	D.	A.	D.	A	D
						Deaths.								
September 1913 ...	1st ...	46,990	...	...	...	7	309	46,674	...	...	6	7	...	...
	2nd ...	46,983	...	...	...	10	4,445	42,523	...	...	14	10	...	...
	3rd ...	46,973	...	...	...	12	7,065	39,896	4	1	22	11	...	...
	4th ...	46,961	...	...	1,500	20	8,610	36,831	2	2	20	18	1	...
October ...	5th ...	45,441	...	...	2,000	9	10,190	33,242	5	...	17	9	4	...
	6th ...	43,432	...	...	500	17	11,575	31,340	4	1	21	16	3	1
	7th ...	42,015	...	...	1,500	21	13,956	27,438	1	...	29	21	...	...
	8th ...	41,394	...	...	1,000	47	17,179	23,168	8	3	53	44	6	1
November ...	9th ...	40,347	...	...	500	64	19,055	20,128	7	6	82	58	6	6
	10th ...	39,783	3,500	11,000	1,000	88	24,818	28,427	10	3	42	35	9	1
	11th ...	53,245	...	...	...	26	26,906	26,313	5	4	30	22	5	3
	12th ...	53,219	...	...	1,000	36	27,987	24,196	4	2	57	34	3	2
	13th ...	52,183	...	...	...	44	29,213	22,926	10	3	67	41	9	3
December ...	14th ...	52,139	...	...	...	38	30,712	21,389	7	2	62	36	6	3
	15th ...	52,101	...	...	...	29	32,490	19,592	11	2	37	27	8	1
	16th ...	52,072	...	...	...	20	34,492	17,560	12	...	34	20	10	...
	17th ...	52,052	...	...	...	18	35,656	16,378	8	3	23	15	7	1
January 1914 ...	18th ...	52,034	...	...	...	17	36,803	15,214	8	3	24	14	6	3
	19th ...	52,017	...	...	...	18	37697	14,302	9	7	18	11	7	7
	20th ...	51,999	...	...	...	17	38,592	13,390	14	6	18	11	13	6
	21st ...	51,982	...	...	...	12	40,509	11,461	5	2	6	10	5	2
	22nd ...	51,970	...	...	...	9	42,280	9,681	4	1	13	8	2	1
February ...	23rd ...	51,961	...	...	...	10	43,596	8,355	4	...	8	10	1	...
	24th ...	51,951	...	...	...	7	44,360	7,584	6	3	3	4	5	2
	25th ...	51,944	...	...	...	2	44,853	7,089	2	2	1	...	2	2
						Total 25 weeks ...	663,948	565,092	150	56	707	392	118	45
						Weekly average population for the period ...	26,55	22,604	...	...	...	...	...	....
						Ratio per mille of the average strength for the period ...	...	...	5·64	2·1	31·28	21·76	4·44	1·69

"With regard to these conditions (1) reduction by deaths can be more or less relied upon as correct; (2) reduction by compulsory evacuation is necessarily treated as a rough and therefore inaccurate estimate. The actual number of persons who left the town week by week cannot possibly be correctly stated. The figure quoted in the tables are based upon the systematic and compulsory evacuation of houses and whole streets which was periodically enforced and the number of persons so evicted has been calculated on the population of each street or block as recorded in the 1911 census. This has been the only possible way of arriving at the variations of the population of the town week by week. (3) Increase by reoccupation has been shown in the 10th week of the inoculation campaign when houses were allowed to be reoccupied by their owners who had previously been compulsorily evicted. These figures are of course not accurate; they are but an average or rough estimate. (4) The increase by extension of the municipal area shown in the 10th week was due to the inclusion of three important hamlets which were infected with plague and where inoculation was also carried on."

It is interesting to note that the numb er of inoculated nearly equalled the number of uninoculated in the 11th week of the campaign.

The average weekly inoculated population may be stated as approximately 26,558 while the similar uninoculated population may be placed at 22,604. The ratio per mille of attacks and deaths calculated on the average population shows that among the inoculated the figures are 5·64 attacks and 2·1 deaths while among the uninoculated the figures are 31·28 attacks and 21·76 deaths. The number of attacks among the uninoculated was therefore five and a half times greater than among the inoculated, while the deaths among uninoculated were ten times more numerous than among the inoculated. There can be little doubt that this extensive inoculation campaign in Coimbatore town saved many lives.

These figures should serve to show that inoculation is a useful means of combating plague and that by this means the mortality from this disease can be considerably reduced. They also show that the quality of the vaccine at present turned out from the Laboratory maintains a high standard of efficiency.

(*b*) Research Work.

I.

Hydrocyanic Acid Gas as an agent for destroying rats and fleas.

5. As stated in the Annual Report of 1912, the governing body of the Indian Research Fund Association gave a grant in the commencement of 1913 to meet the cost of further experiments on fumigation by Hydrocyanic Acid Gas as a preventive measure against the spread of plague. As a preliminary experiment we completely filled one of our small rat-proof experimental godowns with bags of rice and allowed rats to run free among these bags as they might in a grain store. It was observed that after fumigation no dead rats were found within the grain bags but all between the bags or at the corner of the room. The result of the experiments was very encouraging as all the rats were killed.

It was also concluded from certain experiments that fleas in bulk rice and presumably therefore in rice bags do not burrow deeply enough into the rice to escape destruction by the gas. The gas was found not to penetrate into the bags of rice farther than 2 or 3 inches from the surface.

In these experiments the gas was generated by the action of dilute sulphuric acid on lumps of potassium cyanide. With the object in view of prolonging the generation of the gas it was decided to mix together solutions of potassium cyanide and sulphuric acid of 50 per cent. strength. The rate of mixing and therefore the quantity of the gas generated was regulated according to the desire of the operator.

This method was used for fumigating a barge with a hold capacity of 12,000 cubic feet. A large number of rats and fleas were placed in various situations in the holds. Some of the rats were allowed to run loose, and others were placed in cages both above and below the wooden boarding which formed the

floor of the hold. It was found that using the quantity of potassium cyanide we considered sufficient, ½ oz. per 100 cubic feet, all rats and fleas above the floors were killed in one hour, but that, of the rats placed in cages below the boards, or which, running loose, had found their way there, a considerable number survived this period of exposure to the gas; when the period during which the hatches were kept closed was increased to four hours practically all the rats were killed.

II.

Grain bags and clothing as media for the transference of infected rat fleas from one place to another.

6. Experiments were devised to determine to what extent clothes and bedding, on the one hand, and grain bags, on the other, carry the infection of plague. The question resolves itself into whether infected fleas are more readily carried in clothing or in grain.

The method adopted was to start an epizootic in a godown into which a plentiful supply of fleas had been introduced along with bundles of old clothing or gunny bags half full of rice, as the case might be. Subsequently the clothing or gunny bags were introduced into the flea-free godown in which susceptible rats had been placed and observations were made as to whether plague spread among them. The result of these experiments generally is that while it was easy to transfer the infected fleas by means of clothing, in no case were we able to carry the infection in the grain bags. These experiments must however be amplified before any definite conclusion can be arrived at.

III.

The treatment of plague by Iodine.

7. Experiments were made to test the efficiency of the treatment of plague cases by tincture of iodine when administered (*a*) by giving five drops every three hours by the mouth while iodine was applied locally to the buboes; and (*b*) by injecting seven minims of the tincture in saline solution intravenously once every twenty-four hours as advocated by Captain Powel Connor, I.M.S. These methods of treatment were compared with that ordinarily adopted by Dr. Choksey in the Maratha Plague Hospital.

Clinically the cases were recognised to be undoubted cases of plague by Dr. Choksey and with a view to determining the severity of the disease in each case, a bacteriological examination of the blood was made and the cases classified into four groups as we found: (1) no plague bacilli present in one quarter of a cubic centimeter of blood, (2) some but less than ten plague bacilli present in one quarter of a cubic centimeter of blood, (3) more than ten but less than one hundred bacilli in one quarter of a cubic centimeter of blood, and (4) more than one hundred plague bacilli in one quarter of a cubic centimeter of blood.

The result of the examination of the blood of a large number of plague patients (more than 500) by this method had previously shown that all persons with a larger number of plague bacilli than ten in one quarter of a cubic centimeter of blood invariably die while a proportion of the cases with no plague bacilli or less than ten plague bacilli in one cubic centimeter of their blood recover. This classification of cases is necessary when estimating the value of any form of treatment for plague.

In the present series there were sixty cases of plague. Twenty of these cases received the ordinary treatment adopted by Dr. Choksey at the Maratha Plague Hospital, twenty were treated, as described above, with tincture of iodine by the mouth, while twenty others were treated by the same drug administered intravenously.

The table below shows the classification of the cases and the result of treatment in each group of cases. The table shows that of the twenty patients on ordinary treatment (the controls) four recovered and sixteen died. In this series of cases eleven were placed after bacteriological examination of their blood taken

at the time of their admission to hospital in one or other of the invariably fatal groups, while nine were classified in the groups in which recovery was possible. Seven of these latter cases showed no plague bacilli in one quarter of a cubic centimeter of their blood.

Table showing the result of treatment and classification of cases.

Kind of treatment adopted.	Cases with no bacilli in ·25 c. c. of blood.		Cases with some bacilli but less than 10 in ·25 c. c. of blood.		Cases with more than ten but less than 100 bacilli in ·25 c. c. of blood.		Cases with more than 100 bacilli in ·25 c. c. of blood.		Total treated.	
	Recovered.	Died.	Recovered.	Died.	Recovered.	Died.	Recovered.	Died.	Recovered.	Died.
Controls on ordinary treatment...	3	4	1	1	...	3	...	8	4	16
Iodine administered by the mouth.	6	3	2	1	...	1	...	7	8	12
Iodine administered intravenously.	3	4	...	3	...	3	...	7	3	17
Total ...	12	11	3	5	...	7	...	22	15	45

Of the patients treated with iodine by the mouth, twelve died and eight recovered; but in this series, there were only eight cases placed in the fatal groups. While as many as nine showed no bacilli in their blood at the time of admission.

Of the patients who received tincture of iodine by intravenous injections, seventeen died and three recovered; but in this series ten were classed in the fatal groups while only seven showed no bacilli in their blood at the time of admission to hospital.

Excluding the invariably fatal cases with many plague bacilli in their blood before treatment was begun, we have four recoveries out of nine cases among the control cases, eight recoveries out of twelve cases treated by iodine by the mouth and three recoveries out of ten cases by iodine intravenously.

The number of cases treated in each series is not sufficiently large to allow of any definite conclusion to be made regarding the comparative efficiency of any of the methods of treatment. It will be observed that while the cases which recovered after treatment with iodine by the mouth were more numerous, the number which recovered after the intravenous injection of iodine were less numerous than those treated by Dr. Choksey's method.

The number of cases treated is however large enough to show that the simple treatment recommended by the Salvation Army authorities has not yielded in our hands so high a percentage of recoveries as the cases reported by Commissioner Booth Tucker in the 'Pioneer' of the 4th May 1913. This may be due to the fact that firstly our cases generally came under treatment at least twenty-four hours after the onset of the symptoms of the disease, because the patients had to be sent to the hospital and secondly because all our cases were undoubtedly severe forms of the disease, they were all so seriously ill that their transfer to hospital was necessary. It is well known that in the presence of an epidemic of plague, many persons suffer from enlarged and tender glands. These persons often present no other symptoms of the disease and they generally speedily recover. No cases of this type were included in our series but some mild cases of this nature may have been included in the series reported in the 'Pioneer' by Commissioner Booth Tucker. In short, the cases treated by us were all suffering from a severe form of the disease and treatment was generally begun at least twenty-four hours after, generally a longer period after, the onset of the symptoms.

The cases reported by the Salvation Army authorities probably included among them some mild cases, and it appears that treatment was in all cases begun early after the onset of the symptoms.

(*c*) Assistance and Advice rendered.

Examination of rats for plague.

8. The Health Department of the City of Bombay sent daily for examination all rats found dead or caught alive within the bounds of the Municipality. As a note is received with the rats indicating where each rat has been found, it is possible to inform the Health Officer of the City where plague-infected specimens have been picked up, he was thus able to adopt suitable measures to observe and check the progress of the disease throughout the city. During the year 654,516 rats were received at the Laboratory from the Health Department; 438,932 were alive when received, and 215,584 were dead; of the latter 146,478 were examined and among them 7,805 were found to be plague-infected.

The following table shows the progress of plague each half month among men and rats in Bombay. The figures which refer to men record the mortality from plague; the figures showing the progress of the disease in rats are given separately for *Mus rattus* and *Mus decumanus*. These figures have been calculated, because the rats are not examined at the Laboratory on every day of the week, no examination being made on Sundays and Holidays; from the actual figures obtained on the days on which examinations were made a figure has been calculated for all the days of each half month and these figures are recorded:—

Return of rats examined during the year 1913.

	Human plague mortality.		Plague-infected *rattus*.		Plague-infected *decumanus*.	
	Number of deaths from plague.	Percentage above and below mean for the year.	Figures calculated for each half month.	Percentage above and below mean for the year.	Figures calculated for each half month.	Percentage above and below mean for the year.
1913.						
1st to 15th January ...	17	— 84	13.7	— 76	145·0	— 58
16th to 31st January ...	23	— 79	17·1	— 70	225·1	— 35
1st to 15th February ...	50	— 53	35·5	— 39	331·2	— 4
16th to 28th February ...	71	— 34	85·8	+ 48	497·9	+ 44
1st to 15th March ...	168	+ 57	166·2	+188	852·5	+146
16th to 31st March ...	282	+164	196·4	+240	1,133·1	+227
1st to 15th April ...	415	+288	131·2	+127	1,082·5	+213
16th to 30th April ...	456	+326	188·1	+225	933·5	+170
1st to 15th May ...	407	+280	109·6	+ 90	748·8	+116
16th to 31st May ...	321	+200	62·9	+ 9	476·6	+ 38
1st to 15th June ...	78	— 27	20·5	— 65	237·3	— 31
16th to 30th June ...	59	— 45	26·2	— 55	225·0	— 35
1st to 15th July ...	36	— 66	28·8	— 50	189·2	— 45
16th to 31st July ...	40	— 63	41·1	— 29	187·4	— 46
1st to 15th August ...	32	— 70	24·2	— 58	140·8	— 59
16th to 31st August ...	25	— 77	18·7	— 68	102·7	— 70
1st to 15th September ...	17	— 84	10·9	— 81	68·2	— 80
16th to 30th September ...	24	— 78	20·0	— 65	65·0	— 81
1st to 15th October ...	17	— 84	30·0	— 48	52·5	— 85
16th to 31st October ...	15	— 86	10·3	— 82	33·1	— 90
1st to 15th November ...	15	— 86	27·5	— 52	95.0	— 73
16th to 30th November ...	4	— 96	18·7	— 68	68·7	— 80
1st to 15th December ...	4	— 96	21·2	— 63	147·5	— 57
16th to 31st December ...	3	— 97	82·7	+ 43	270·7	— 22

(*d*) Educational Work.

9. A number of medical gentlemen have attended the Laboratory for practical instruction in the examination of rats with a view to making a diagnosis as to whether the rats are plague-infected or not. Other gentlemen, among them many distinguished visitors, have been shown the methods employed in the manufacture of anti-plague vaccine. A few medical men have gone through a course of instruction in the technique of inoculation.

PART II.

Work of the Laboratory as the Provincial Bacteriological Laboratory.

(*a*) Routine Work.

I

Examinations of Pathological Fluids and Discharges, Waters, etc.

Pathological work done at the Laboratory.

10. A large number of specimens were received for examination.

I.—Blood Sera :—

Of the 180 specimens examined, 59 gave a positive agglutinative reaction to Bacillus typhosus and 2 to Bacillus paratyphosus B.

II.—Blood Smears :—

Of numerous specimens examined, malarial parasites were present in 9 cases. Differential counts were made 25 times.

III.—Sputa :—

32 specimens showed the presence of Tubercle bacilli.

IV.—

4 specimens showed the presence of Lepra bacilli.

V.—Throat Discharges :—

Bacillus Diphtheriæ was found in one specimen.

VI.—Pus :—

6 cases showed the presence of Gonococci.

VII.—Faeces and Urine :—

18 specimens of faeces were examined and 63 of urine for various pathological conditions.

VIII.—Sera for the Wassermann reaction :—

This test was performed 142 times with 79 positive reactions.

IX.—Water :—

19 specimens were examined bacteriologically.

X.—*Post mortem* examinations were held on 86 animals received for the most part from the Zoological Gardens, Bombay, to ascertain the cause of death.

XI.—Three snakes, 2 worms, 40 mosquitos, and a fish were identified.

XII.—In two cases blood smears from horses showed the presence of Trypanosomes.

XIII.—Various other organisms were identified from specimens examined which it would be no advantage to detail.

II.

Diagnosis of Rabies.

Examination of dogs for Rabies.

11. The examination of brains of dogs and other animals for the diagnosis of rabies was performed in 96 cases. The brains were received from the following places :—

Bombay—			
Through the Bombay Veterinary College :—			
Dogs	56		
Goats	1		
		57	
Private owners		9	
Bombay Police		15	

Outstations—

Poona	...	5
Surat	...	2
Broach	...	1
Ajmere	...	1
Kolhapur	...	1
Karwar	...	1
Násik	...	2
Sátára	...	1
		14

Of these 96 cases, 58 gave a positive result, and 28 a negative and in 10 it was impossible, on account of the poor preservation of the brain material, to give any opinion.

Wherever it was possible smears from the fresh brain as well as sections of the hardened brain were made. Seventy-one combined examinations were thus made and in all but 2, where the smear result was doubtful, the results were parallel. The reliability of the smear examination is thus proved and one is often enabled to give a definite opinion within a few minutes of receiving the material.

The necessity of carefully investigating the clinical symptoms of the supposed rabid animals before death was brought out in one case where, although the microscopic examination gave a negative result, the persons bitten were advised to go to a Pasteur Institute for treatment on account of the suspicious symptoms of the dog which had bitten them. An animal experiment gave a positive result.

One can therefore alone from the microscopic findings make a diagnosis of rabies, but the clinical symptoms during life and the *post mortem* appearances must also be taken into account to justify a diagnosis of freedom from rabies.

III.

Pathological tissues received for diagnosis.

12. During the year sections were cut and specimens examined in 316 cases. The following table shows the tumours and other morbid tissues examined at the Laboratory during the year 1913 :—

Classification of morbid tissues sent for examination.

I.—Tumours—

A.—Non-malignant Tumours—

1.	Fibroma ...	19
2.	Neuro-fibroma ...	1
3.	Myxoma ...	1
4.	Adenoma ...	8
5.	Haemangioma ...	6
6.	Lymphangioma ...	1
7.	Papilloma ...	8
8.	Lipoma ...	1
9.	Fibro-myoma ...	2
10.	Adeno-myoma ...	1
11.	Chondroma ...	2
12.	Cystoma ...	6
		56

B.—Malignant Tumours—

(*a*)	1.	Carcinoma glandularis simplex ...	10
	2.	„ scirrhus ...	4
	3.	„ encephaloid ...	5
	4.	„ adeno ...	23
	5.	Epithelioma ...	36
	6.	Basal-celled epithelioma ...	2
	7.	Columnar-celled carcinoma ...	1
	8.	Melanotic carcinoma ...	1
	9.	Rodent ulcer ...	1
			83

(*b*) Sarcomata—			
1. Round-celled sarcoma	... 14		
2. Spindle-celled „	... 5		
3. Mixed-celled „	... 4		
4. Myxo-sarcoma	... 2		
5. Myeloid sarcoma	... 6		
6. Melanotic sarcoma	... 3		
		34	
(*c*) Perithelioma	...	2	
(*d*) Endothelioma	...	6	
(*e*) Malignant papillary adenoma	...	4	
II.—Granulomata—			
1. Tuberculous	... 14		
2. Syphilitic	... 6		
3. Other granulomata	... 8		
		28	
III.—Inflammatory Processes, etc.—			
1. Chronic Inflammatory condition ...	... 12		
2. Acute	... 2		
3. Ulcers simple	... 2		
4. Lymph adenoma	... 4		
5. Polypus	... 2		
6. Mycetoma (white variety)	... 2		
7. Streptothricosis	... 1		
8. Leprosy	... 2		
9. Uterine scrapings (endometritis, etc.) ...	... 8		
10. Pathological conditions of various organs	... 33		
		68	
IV.—Tissues found to be normal		22	
V.—Diagnosis impossible owing to the poor preservation of tissues or for some other reasons		13	

IV.

Snakes.

Snakes.

13. Poisonous Indian snakes are kept in the laboratory for the purpose of obtaining a supply of venom for the manufacture of anti-venomous serum. The following table shows the number of snakes received during the year :—

Places.	Cobra.	Russell's v per.	Krait.	Total.
Bombay Presidency	12	20	1	33
Central Provinces	1	...	...	1
Rájputána	2	...	...	2
Total ...	15	20	1	36

(*b*) Research work.

I.

The distribution of Guineaworm disease in India.

14. It is impossible to define accurately the distribution of guineaworm disease over India because of the want of data concerning the prevalence of this disease. The only official figures available dealing with this disease are those recorded in the Annual Reports of the Sanitary Commissioner with the Government of India which pertain to Native Troops and Prisoners; even in these reports the figures for the troops have been discontinued since 1908. From a study of these statistics Dr. Turkhud has come to some interesting conclusions regarding the distribution of this disease which is very widely disseminated throughout India.

Geographical areas.

For the purpose of collecting medical statistics, and also for the tabulation of meteorological data for the medical department, the whole of India has been divided into 11 geographical groups which are so arranged as to "represent areas which are fairly homogenous so far as the chief prevailing diseases and climatic conditions are concerned". These groups are :—

I.—Burma Coast and Bay Islands.
II.—Burma Inland.
III.—Assam.
IV.—Bengal and Orissa.
V.—Gangetic Plain and Chutia Nagpur.
VI.—Upper Sub-Himalaya.
VII.—North-West Frontier, Indus Valley and North-Western Rájputána.
VIII.—South-Eastern Rájputána, Central India and Gujarát.
IX.—Deccan.
X.—Western Coast.
XI.—Southern India.

The distribution of the disease among Indian Troops stationed in certain geographical areas.

The division in which dracontiasis is most prevalent among native troops is south-eastern Rájputána, Central India and Gujarát. Here the disease prevails to the extent of 18 cases per mille of the average strength per annum. A ratio of 13·4 per mille is found in Gujarát. The other divisions where the disease prevails are :—

Deccan Proper,	ratio 7·1	per mille of average strength.
Western Coast,	„ 5·72	do.
Gangetic Plain,	„ 5·5	do.
Upper Sub-Himalaya,	„ 5·22	do.
Sind,	„ 5·12	do.

While in these divisions the disease is common, all the hill stations appear to be free from it except those situated in the North-West Frontier Province. Inland Burma also appears to be free from the disease.

The distribution of the disease among prisoners.

The incidence among prisoners in jails probably more accurately represents the prevalence of the disease in the various parts of India, for the majority of prisoners live under normal Indian conditions in the same districts in which the jails are situated in which they are confined. Judged by the incidence of the disease among prisoners, the western part of the Madras Presidency is the most afflicted area (39·64 of average annual strength per mille). The division which in intensity of infection follows the western portion of the Madras Presidency is the Deccan with the very high ratio of 28·10 per mille of average annual strength. The other localities where dracontiasis is common are :—

The Northern Circars,	ratio 12·09	per mille.
Madras and Carnátic,	„ 11·86	do.
Gujarát and Káthiáwár,	„ 10·66	do.
The Western Coast,	„ 10·53	do.
The Indus Valley and North-Western Rájputána,	„ 10·11	do.

The Hill Stations, Burma (Inland and Coast) and the Andamans are all free from the disease.

The distribution of the disease among prisoners, a more reliable guide to the extent and distribution of the disease among the general population than that known to exist among Indian Troops.

The high ratio among the sepoys stationed in a locality showing a low ratio among the prisoners may perhaps be explained on the ground that the sepoys contracted infection in their native villages while on leave or before their transfer to the stations in which they were reported to be sick with the disease. Again a low ratio among the troops in the localities where the disease is prevalent among the prisoners, may be attributed to the superior sanitary surroundings in which the sepoys dwell.

II.

15. Some interesting observations were made in the village of Sarsola near Thána on the incidence of guineaworm disease by Major Glen Liston, I. M. S., and Dr. Turkhud, M.B., C.M. (Edin.). With the assistance of Mr. Bhave, a graduate in science, and an influential Indian gentleman residing near the village, a census of the inhabitants was taken. It was found that 10·78 per cent. of the villagers had suffered during the current year from the disease and that approximately 39 per cent. of the villagers had at one time or another suffered from the disease. Of the whole population the males were affected in the proportion of 43 per cent., while 26 per cent. of females suffered. The source of the disease was traced to an old well belonging to the villagers. The well contained numerous cyclops, of which 38·6 per cent. harboured young guineaworms, as ascertained at the first examination. Although instruction was given to the villagers regarding the precautions which ought to be taken to avoid the disease, and attempts were made by Mr. Bhave to assist them in securing pure drinking water supply, owing to the suspicion and indifference of the population no attempt to adopt remedial measures had been taken several months later when their village was re-visited.

The extent of the Guineaworm disease in Sarsola village with an account of how the disease is spread in that village.

In addition to the visits paid to Sarsola, excursions were made to Kadav and Takve in Kolába district, Yeola and adjoining villages in Násik district, and Shetbunder (Elephanta). In these villages guineaworm disease is very common and our observations made at these places confirmed our views that ignorance, indifference and prejudice on the part of the people are the chief causes which favour the spread of the disease. The parapets of wells are allowed to remain in a state of disrepair so that surface water, after being fouled by bathing and washing operations which are promiscuously carried on near village wells, easily finds its way back into the wells. It is a common sight to see the village wells, the water of which should be scrupulously guarded against contamination, being utilised as swimming ponds, and where a well is provided with steps, to see people washing themselves in the well water. It seems likely, that although this disease is one of the most easily prevented of all diseases we know, little progress will be made in eradicating it from the country till the people of themselves demand a higher standard in the purity of their drinking water; so long as they are content to drink water which has been used for bathing purposes the disease is likely to prevail.

The presence of the disease in other villages and the cause of its existence in them.

III.

16. Experiments made on monkeys in the Laboratory to ascertain the mode of transmission of the disease gave the following result. Two series of experiments were carried out. In the first series various methods were employed for bringing about infection. Thirteen monkeys were used, some of them were fed by the mouth on infected cyclops and some with living guineaworm embryos, while others were injected subcutaneously with the blister fluid containing living embryos, or with embryos suspended in normal saline. *Post mortem* examinations on these monkeys were carried out after intervals varying from one month to one and a half years from the date of experiment. The examinations were conducted very carefully but the results in all instances were negative. The monkeys injected subcutaneously showed no *local lesions*, and in none of them were any guineaworms found.

Experiments to determine the method of infection by guineaworm. Monkeys apparently not susceptible to infection.

In the month of April 1913, a sample of water obtained from the well at Sarsola showed the presence of a large number of cyclops harbouring guineaworm embryos. About 38·6 per cent. of the cyclops were found infected with guineaworm embryos. Advantage was taken of this, and a second series of experiments was instituted on twelve monkeys which were fed on this naturally infected well water, each monkey being given about 100 c. c. of the water through a stomach tube. More than 100 cyclops were present in 100 c. c. of water, so that each monkey swallowed about 38 infected cyclops.

Nine of these monkeys were examined at periods varying from one to ten and a half months after the date of feeding. Careful *post mortem* examinations were made but the results were negative in all the animals examined. The remaining three monkeys out of the series are still alive and well. The monkeys used were all *Macacus sinicus*.

Successful experimental infection of man by guineaworm: the disease produced by swallowing infected cyclops.

Owing to our failure to infect monkeys and the observed absence of the disease among the animals living in infected villages, we thought that the worm might only develop in man; volunteers were therefore called upon to drink water containing infected cyclops. As stated in our last report, five persons came forward. Each volunteer was given five infected cyclops to swallow in a small quantity of sterile water. The experiment was inaugurated on the 5th of April, and the cyclops used had been infected on the 24th of March 1913. On the 18th March 1914, *i. e.*, three hundred and forty-eight days after drinking the infected water, one of the volunteers a Laboratory assistant, developed a very small blister on the dorsum of his right foot. The appearnace of the blister was accompanied with fever, vomiting and diarrhœa. On the 30th, the blister had assumed the size of a marble and it was then found to contain guineaworm embryos. From this date large number of embryos were daily collected from the worm until the 14th April. The worm then broke; some local swelling and suppuration supervened, but this eventually subsided. In this case the volunteer, with the exception of two or three little ulcerations about the body in the rainy season, showed absolutely no symptoms until the appearance of the blister, nearly a year after he swallowed the infected cyclops.

The other volunteers showed no signs and symptoms of the disease with the exception of a little occasional urticaria. In the case of one of the volunteers the blood examination showed an eosinophilia to the extent of 34 per cent. in the month of October 1913, but he did not develop a guineaworm.

These experiments go to prove that the cyclops is the actual intermediary host for the guineaworm, that the monkey *Macacus sinicus* is probably naturally immune to the disease, and that man is to some extent resistent to infection, for only one person of five who swallowed cyclops containing six to eight guineaworm embryos developed the disease.

IV.

Number of young produced by a single guineaworm.

17. An attempt was made to ascertain the number of embryos contained in an adult worm. This was done by dissecting out the uterus from a worm and counting the embryos in a measured volume of the fluid found in the uterus and thus estimating the total number contained in the uterus. The result showed that a mature guineaworm contains about three million embryos.

V.

Experiments with potassium permanganate to test its action in cyclops, the intermediary host of the guineaworm.

18. The following experiments were conducted to ascertain the efficacy of potassium permanganate for destroying cyclops:—

(1) To a large bottle of well water from Sarsola containing many cyclops a quantity of permanganate of potassium in solution was added until the water assumed a pink colour. Two and a half hours afterwards all the cyclops were dead. The bottle was put aside in the laboratory: nine days later some living cyclops were again seen in the water.

(2) A standard solution of potassium permanganate containing 3·95 grammes to the litre was first prepared and some of this solution in quantities varying from 1 c. c. to 100 c. c. was added to a litre of water. Six cyclops were transferred to the water containing different strengths of potassium permanganate. The experiment showed that when 100 c. c. of the standard solution was added to a litre of water, all the six cyclops were killed in an hour. When 75 c. c. of the standard solution was added all but two cyclops

were killed in one hour and six cylcops were dead in one and a half hours. When 25 c. c. and 50 c. c. of the solution was added, all cyclops were dead in one and a half hours, but with 20 c. c., 15 c. c., 10 c. c., 8 c. c. and 6 c. c., to the litre of water some cyclops were still alive after three hours. With 4 c. c. and lesser quantities of the standard solution some of the cyclops survived even after three hours exposure but all were dead in 24 hours.

(3) Lastly, the well at Sarsola itself was disinfected on 16th March 1914 with nearly three lbs. of potassium permanganate so that a distinct pink tint was imparted to the water. On the 23rd March the water showed no cyclops. On the 30th of that month, however, it was found to contain a large number of daphnia and also a few cyclops. On the 8th April, the number of cyclops had increased, and in addition to daphnia, a few larvæ of Anopheles rossi were found. On this date 5·9 per cent. of the cyclops were found infected. By the 20th of that month the number of cyclops had considerably increased and the percentage of infection among them had risen to 14 per cent. Daphnia and Anopheles larvæ were still present.

Potassium permanganate destroys adult cyclops but not their eggs so that after a time cyclops reappear in the water and these cyclops in favourable circumstances soon become infected with young guineaworms.

Summary.

Our researches on guineaworm disease have shown that:—

(1) The disease is widely prevalent in India; in some parts of the country, as for example the western portion of the Madras Presidency, 39 persons per mille of the population annually suffer from the disease if prisoners admitted to jails represent the extent to which the disease exists among the general population. Even in the Deccan 28 persons per mille harbour the worm and in some villages as many as ten per cent. of the inhabitants may be affected in a single year.

(2) Males appear to suffer from the disease more frequently than females. It does mot occur among domestic animals living in infected villages nor can the disease be conveyed in the laboratory to monkeys (*Macacus Sinicus*). Even man is to some extent resistent to infection for only one of five persons who swallowed in water from six to eight guinea-worm embryos contained within cyclops developed the worm.

(3) The worm appears under the skin nearly a year after drinking infected water; during this long period the infected person shows no symptoms of the disease.

(4) The worm is very prolific, each female produces about three million young worms.

(5) No other intermediary host for the young guineaworm than cyclops was found; the usual mode of infection is by drinking water containing infected cyclops.

(6) Cyclops were readily killed in weak solution of Potassium permanganate but they reappear in the water after a time having probably developed from eggs which resist the action of this drug. After disinfection with Potassium permanganate, wells may become infected again within a month if proper care is not taken to prevent reinfection. Cyclops can be easily removed from water by merely passing it through a piece of fine cloth.

(7) The existence of this easily prevented disease in India is due largely to the indifference, ignorance and prejudices of the people who are careless about the condition of their wells and do not mind drinking water which has been soiled by people bathing in it.

VI.

19. In the Annual Report for 1912 a short summary appeared of a study of cholera and cholera-like vibrios isolated in Bombay by Captain Gloster, I. M. S., in the summer of 1912.

Experiments with cholera and cholera-like vibrios.

A more detailed account was given in a paper read before the All-India Sanitary Conference at Madras in 1912.

In this paper it was pointed out that the investigations of Lamb and Haffkine in India and many other observers elsewhere have shown that there is an epidemiological relationship between cholera-like vibrios and true cholera vibrios, the former being found in water and stools chiefly in association with epidemic cholera. A study of the morphological and cultural characters of the Bombay vibrios showed that these characters could not be relied on to differentiate the cholera-like from the true cholera strains.

Cholera and cholera-like vibrios are often associated together or found independently during the prevalence of a cholera epidemic.

The distinction between the two groups of vibrios was based solely on their behaviour with a standard agglutinating cholera serum (Kolle's).

Cholera vibrios differentiated from cholera-like vibrios only by agglutination with specific serum in high dilution.

All the vibrios which were completely agglutinated in dilutions of this serum of 1—1,000 or upwards were classed as cholera vibrios. This group was sharply distinguished from the other group of cholera-like vibrios none of which were agglutinated in the dilutions of the standard serum above 1—100.

During the year under report a more complete study has been made of the serum reactions of the vibrios with the object of determining what relationship, if any, exists between vibrios of the cholera-like group and true cholera vibrios and between the different vibrios of the cholera-like group. Further experiments with regard to their hæmolytic properties and their behaviour in concentrated solutions of Magnesium suphate have also been carried out.

A list of the vibrios examined is given in Table No. I. Most of them were isolated in 1912 but a few older strains are included. All had been repeatedly subcultured on agar at intervals of a fortnight.

TABLE No. I.

Cholera and Cholera-like vibrios.

Number.	Name.	Date.	Origin.	Remarks.
		Cholera vibrios.		
		1912.		
1	1091 ...	June 21 ...	Stool ...	Typical cholera.
2	1092 ...	June 21 ...	D	Do.
3	1407 ...	June ...	Do. ...	Do.
4	1190 ...	July 5 ...	Do. ...	Do.
5	1255 ...	July 25 ...	Do. ...	Do.
6	1259 ...	July 25 ...	Do. ...	Do.
7	No. 3 ...	July 25 ...	Do. ...	Do.
8	DeSouza ...	August 20 ...	Do. ...	Do.
9	Billimoria ...	August 22 ...	Do. ...	Do.
10	Nusserwanji I ...	August 20 ...	Do. ...	Do.
11	Delaney ...	August 23 ...	Do. ...	Do.
12	Dossabhoy ...	September 9 ...	Contents of ileum.	Do.
		1913.		
13	E. G. Hospital ...	October 1 ...	Stool ...	Do.
14	Bombay ...	.....	Do. ...	Do.
15	L. I. P. M. ...	.....	.....	Do.
16	Yeravda ...	.....	.....	Do.
		Cholera-like vibrios.		
		1912.		
18	1406 ...	June ...	Water ...	Well exposed to fæcal contamination.
19	1507 ...	Do. ...	Do. ...	Do.

Number.	Name.	Date.	Origin.	Remarks.
		Cholera-like vibrios —continued. 1913.		
20	1280 ...	August 8 ...	Stool ...	Convalescent case of cholera.
21	1299 ...	August 14 ...	Do. ...	Do.
22	DeLisle Road ...	August 10 ...	Water ...	Tank exposed to fæcal contamination.
23	Jehangir ...	September 6 ...	Stool ...	Case of cholera.
24	1370 ...	September 7 ...	Do. ...	Mild case of cholera.
25	Sewage ...	September 10 ...	Sewage ...	From drain receiving ejecta of cholera cases.
26	Cockroach ...	September 10 ...	Cockroaches ...	Contained in sewage V., No. 26.
27	Nusserwanji ...	September 6 ...	Stool ...	From same stool as No. 10; isolated 16 days after stool was passed.

Agglutination.

Cross agglutination experiments with sera prepared from a number of cholera and cholera-like organisms.

Sera were prepared from 15 cholera and 10 cholera-like strains by giving rabbits a series of 4 to 5 injections of suspensions of young agar cultures. A complete series of cross agglutination experiments were carried out.

Further as control tests, all the vibrios were put up on four occasions with the sera of normal rabbits and once with the serum of a rabbit which had been immunised against Shiga's dysentery bacillus. The tests were made in Wright's tubes macroscopically and the results noted after 2 to 24 hours.

The result of these agglutination tests may be summarised as follows:—

(1) The sera of normal rabbits and the serum of a rabbit immunised against Shiga's bacillus in dilution of 1—10 and upwards had no agglutinating effect on any of the vibrios.

(2) Each of the 15 sera prepared from cholera vibrios agglutinated all the cholera vibrios.

(3) The agglutinating effect of the cholera serums on the vibrios of the cholera-like group was on the whole not marked. The sera prepared from cholera vibrios Dossabhoy, 1255 and 1259, however, agglutinated vibrio sewage.

(4) The cross agglutination of the vibrios of the cholera-like group showed that only two of these, *viz.*, vibrios 1370 and 1299 are identical. This was confirmed by complement fixation tests.

(5) Several of the sera prepared from cholera-like vibrios agglutinated certain vibrios of the cholera group. In these instances the agglutination was slow and usually not complete under 20—24 hours. For example a serum prepared from vibrio 1406 was put up with cholera vibrio E. G. H. and after 24 hours agglutination was complete in dilutions of the serum up to 1—1,280. The salt control showed no trace of agglutination. The above is only one example of many similar tests.

The agglutination of cholera vibrios by serum prepared from cholera-like organisms is admittedly anomalous and difficult to explain. The experiments were, however, repeated and carefully controlled. It is of interest to consider whether such agglutinations are specific and indicate a group relationship between some of the cholera-like and some of the cholera vibrios. Certain facts lead one to think that the agglutination in these cases is not always a specific one, *e. g.*—

Non-specific agglutinations.

(1) The agglutination was not usually a cross one, *e. g.*, in the example above, vibrio 1406 was only very slightly agglutinated by serum E. G. H.

(2) Some cholera strains, which were most markedly agglutinated, became spontaneous agglutinators later on.

(3) (*a*) A serum prepared from the cholera-like vibrio 1280 agglutinated cholera vibrio E. G. H. partially in dilutions up to 1—1,000 and the homologous vibrio 1280 up to the same limit. The serum was absorbed with vibrio 1280 which removed the agglutinin for 1280 but the serum still retained its power of agglutinating vibrio E. G. H. unimpaired. Another portion of the serum was absorbed with vibrio E. G. H. and, after absorption, the serum agglutinated both vibrios E. G. H. and 1280 to the original limits. The result of the first absorption test shows that serum 1280 does not contain a group agglutinin for vibrio E. G. H. while the failure to remove the 'agglutinin' for vibrio E. G. H. by saturation of the serum with this vibrio suggests that the apparent agglutination of the vibrio by the serum is in this instance a non-specific phenomenon.

Absorption tests.

(*b*) A serum prepared from the cholera-like vibrio sewage agglutinated both the homologous vibrio and the cholera-like vibrio E. G. H. in dilutions up to 1—1,000 in 20 hours. When absorbed with vibrio sewage the agglutinins for both vibrio sewage and vibrio E. G. H. were removed. Absorption with vibrio E. G. H. completely removed the agglutinin for this vibrio but the serum still agglutinated vibrio sewage. This experiment suggests that vibrio sewage contains a group agglutinin for cholera vibrio E. G. H.

Complement fixation.

Experiments to differentiate true cholera from cholera-like vibrios by complement fixation.

The results of the complement deviation tests were very definite with Kolle's agglutinating serum. With this serum all the agglutinating vibrios fixed complement while none of the cholera-like vibrios gave fixation. Cholera-like vibrio sewage, however, gave complement fixation with a serum prepared from the cholera vibrio Dossabhoy.

Hæmolytic properties.

Experiments to differentiate true cholera vibrios from cholera-like vibrios by their hæmolytic action on the red blood corpuscles of sheep.

The hæmolytic properties were tested in a liquid medium. A twenty-hour agar culture was emulsified with 3 c. c. of normal saline of this emulsion, 0·1 c. c. was added to 9 c. c. of normal saline in a small test tube and 1 drop of defibrinated blood was put in each tube. The results were noted 2, 24 and 48 hours, *vide* Table No. II. None of the cholera vibrios hæmolysed sheep's red blood cells even in 48 hours, whereas all the non-cholera vibrios except vibrio cockroach produced complete or nearly complete hæmolysis in 24 hours.

TABLE No. II.

Cholera vibrios.

Serial No.	Vibrio.	Sheep's red blood cells.		
		2 hours.	24 hours.	48 hours.
1	1091	No. H.	No. H.	No. H.
2	1092	"	"	"
3	1407	"	"	"
4	1190	"	"	"
5	1255	"	"	"
6	1259	"	"	"
7	No. 3	"	"	"
8	DeSouza	"	"	"
9	Billimoria	"	"	"
10	Nusserwanji I	"	"	"
11	Delaney	"	"	"
12	Dossahhoy	"	"	"
13	E. G. Hospital	"	"	"
14	Bombay	"	"	"
15	L. I. P. M.	"	"	"
16	Yeravda	"	"	"

Serial No.	Vibrio.	Sheep's red blood cells.		
		2 hours.	24 hours.	48 hours.
	Cholera-like vibrios.			
18	1406	No. H.	N. C. H.	N. C. H.
19	1507	Mod. H.	N. C. H.	N. C. H.
20	1280	C. H.	.....	.....
21	1299	No. H.	N. C. H.	N. C. H.
22	DeLisle Road	N. C. H.	N. C. H.	N. C. H.
23	Jehangir	No. H.	C. H.	.....
24	1370	No. H.	N. C. H.	N. C. H.
25	Sewage	Mod. H	N. C. H.	N. C. H.
26	Cockroach	No. H.	No. H.	No. H.
27	Nusserwanji II	Sl. H.	C. H.	.....
	Salt Control ...	No. H.	No. H.	No. H.

No H — No hæmolysis. C. H. — Complete hæmolysis.

Mod. H. — Moderate hæmolysis. N. C. H. — Nearly complete hæmolysis.

Salting out by Magnesium sulphate.

Experiments to differentiate true cholera from cholera-like vibrios by salting out with Magnesium sulphate.

The technique employed was that described by Major E. D. W. Greig, I. M. S., in the Indian Journal of Medical Research, Vol. I, No. 2, October 1913. The results are given in Table No. III. The readings were taken after half hour at room temperature.

Thirteen out of sixteen cholera vibrios were partially or completely precipitated in 90 per cent. saturation of Magnesium sulphate; out of 10 non-cholera vibrios 2 were precipitated in this concentration.

TABLE No. III.

Serial No.	Name of culture of cholera vibrio.	Reaction with specific agglutinating serum (Kolle's).	Presence or absence of precipitation in saturation percentages of vibrio suspension with $MgSO_4$.					Control with 0·85% NaCl.
			90%	80	70%	60%	50%	
	Cholera Vibrios.							
1	1091	All positive in dilutions 1—1,000 or upwards.	+	+	±	—	—	—
2	1092		+	+	Tr.	—	—	—
3	1190		+	+	Tr.	—	—	—
4	1407		+	±	—	—	—	—
5	1255		+	±	±	±	±	±
6	1259		±	±	±	±	±	±
7	Stool 3		—	—	—	—	—	—
8	Delaney		+	±	Tr.	—	—	—
9	DeSouza		±	Tr.	—	—	—	—
10	Billimoria		—	—	—	—	—	—
11	Nusserwanji I ...		+	±	±	±	—	—
12	Dossabhoy		+	±	Tr.	—	—	—
13	E. G. Hospital ...		—	—	—	—	—	—
14	Bombay		Tr	—	—	—	—	—
15	L. I. P. M.		+	—	—	—	—	—
16	Yeravda		+	±	—	—	—	—

Serial No.	Name of culture of cholera vibrio.	Reaction with specific agglutinating serum (Kolle's).	Presence or absence of precipitation in saturation percentages of vibrio suspension with $MgSO_4$.					Control with 0·85% NaCl.
			90%	80%	70%	60%	50%	
	Like non-Cholera vibrios.							
18	1406	All negative in dilutions 1—100.	—	—	—	—	—	—
19	1507		—	—	—	—	—	—
20	1280		—	—	—	—	—	—
21	1299		—	—	—	—	—	—
22	DeLisle Road... ...		Tr.	—	—	—	—	—
23	Jehangir		—	—	—	—	—	—
24	1370		—	—	—	—	—	—
25	Sewage		+	+	+	—	—	—
26	Cockroach		—	—	—	—	—	—
27	Nusserwanji II ...		—	—	—	—	—	—

\+ Complete precipitation. Tr. Trace of precipitation.
± Marked but not complete precipitation. — No precipitation.

Note on a vibrio isolated from sewage, No. 25, Table I.

A vibrio isolated from sewage which although not a true cholera vibrio, very closely resembled the cholera vibrio in some of its characters.

This vibrio is of interest as it resembles a cholera vibrio in some of its characters.

It was isolated in September 1912 from the contents of a drain in Colaba Asylum which received the discharge from several water closets. A cholera epidemic was going on among the inmates at the time and the closets were used by cholera patients. The vibrio resembled in its morphology and cultural characters other cholera-like and cholera vibrios. It was agglutinated by Kolle's cholera serum in dilutions up to 1—100. After repeated sub-culturing on agar extending over a period of more than a year, its agglutination with the standard serum was not altered. It was agglutinated more powerfully however by some of the serums prepared from Bombay cholera vibrios (Table No. IV).

TABLE No. IV.

Agglutination of vibrio sewage by various serums.

Date.	Serum.	Salt Control.	Serum dilutions.							—
			1—50	1—100	1—500	1—1,000	1—5,000	—	—	
4th Oct. 1912 ...	Kolle's High titre ...	—	+	+	—	—	—	...	...	Result after 20 hours room temperature.
23rd Sept. 1913...	Do. ...	Tr.	+	+	Tr.	Tr.	Tr.	...	...	Do.
			1—20	1—40	1—80	1—160	1—320	1—640	1—1,280	
17th Feb. 1914 ...	Do. ...	Tr.	++	++	++	+	Sl.	Sl.	Sl.	Do.
5th Feb. 1914 ...	Dossabhoy	—	++	++	+	+	+	+	+	Do.
17th Mar. 1914...	E. G. Hospital ...	—	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Do.
18th Mar. 1914...	1255	—	+	+	+	+	—	—	—	Do.
20th Mar. 1914...	1259	—	++	++	++	++	+	Sl.	Sl.	After 2 hours a 37° C.
31st Mar. 1914 ...	Stool 3	Sl.	++	++	+	+	+	+	+	Result after 20 hours room temperature.
1st April 1914 ...	DeSouza	Sl.	++	++	+	Sl.	Sl.	Sl.	Sl.	Do.
8th Jan. 1914 ...	Normal Rabbit ...	—	—	—	—	—	...	...	...	Do.
15th Jan. 1914 ...	Do. ...	—	—	—	—	—	—	—	—	Do.
3rd June 1914 ...	Do. ...	—	—	—	—	—	—	—	—	Do.
14th Feb. 1914 ...	Rabbit immunised with Shiga's bacillus.	—	—	—	—	—	—	—	—	

— No agglutination. + Marked agglutination. ++ Complete agglutination.
Sl. Slight agglutination. Tr. Trace agglutination.

A serum prepared from vibrio sewage had a marked agglutinating effect on many of the cholera vibrios isolated in Bombay (Table No. V). As mentioned above absorption tests seemed to show that this agglutination was due to the serum containing a group agglutinin for these vibrios. Although the vibrio did not fix complement in the presence of the standard cholera serum it gave fixation with a serum prepared from our cholera vibrio Dossabhoy.

TABLE No. V.

Agglutination of cholera vibrios by serum prepared from the cholera-like vibrio sewage.

Date.	Vibrio.	Salt Control.	Dilutions of the serum sewage.							—
			1—20	1—40	1—80	1—160	1—320	1—640	1—1,280	
13th Jan. 1914...	1091	—	++	++	++	Tr.	—	—	—	20 hours result; room temperature.
Do. ...	1092	—	++	+	Sl.	—	—	—	—	Do.
Do. ...	1190	Tr.	++	++	++	+	+	+	+	Do.
Do. ...	1407	—	+	+	Sl.	Tr.	Tr.	—	—	Do.
Do. ...	1255	Tr.	++	++	+	+	+	+	Sl.	Do.
Do. ...	1259	—	++	++	+	+	+	+	Sl.	Do.
Do. ...	No. 3	—	++	++	+	Sl.	Tr.	Tr.	Tr.	Do.
Do. ...	DeSouza	—	Tr.	Tr.	—	—	—	—	—	Do.
Do. ...	Billimoria	Tr.	++	++	++	++	+	+	Sl.	Do.
Do. ...	Dossabhoy	—	+	+	Tr.	—	—	—	—	Do.
Do. ...	L. I. P. M.	—	Sl.	—	—	—	—	—	—	Do.
Do. ...	Yeravda	—	Sl.	—	—	—	—	—	—	Do.
Do. ...	E. G. H.	—	++	++	++	++	++	++	++	Do.

— No agglutination. + Marked agglutination. ++ Complete agglutination.
Sl. Slight agglutination. Tr. Trace agglutination.

In hæmolytic properties for sheep's corpuscles it resembled a 'cholera-like' vibrio.

Finally it was readily salted out in 90 per cent. concentration of Magnesium sulphate resembling in this respect a cholera vibrio, see Table III.

VII.

Bovine Tuberculosis in its relation to human tuberculosis in India.

20. In the annual report last year we stated that an inquiry has been made into the prevalence of tuberculosis among cattle in Bombay. The result of this inquiry showed that tubercle bacilli could not be found in any of the samples of milk examined, nor were we able to discover any evidence of tuberculosis among the cattle slaughtered at the Bandra Slaughter House. The Veterinary Inspector of the Slaughter House stated that during an experience extending over several years he had not seen a single carcass affected with gross tuberculous lesions. We pointed out that this experience in Bombay contrasted with that reported from Cawnpore where among two thousand cattle slaughtered in 1912, 3 per cent. were found to be affected with tuberculosis. Through the kindness of the Health Officer, Cawnpore, we have been favoured with some tissues obtained from cattle slaughtered in Cawnpore which were said to be tuberculous. We have made a microscopical examination of these tissues and found no evidence of tuberculosis. The portions of the lungs sectioned showed catarrhal inflammation and some necrosis but no typical caseation, the cellular characters of the part surrounding the necrotic areas were not those characteristic of tubercle. We are, therefore, constrained to believe that bovine tuberculosis is very rare in India.

In order to ascertain whether the bovine type of tubercle bacillus could be found in the enlarged tubercular glands of children an attempt has been made to cultivate the bacillus from as many cases as possible. The majority of workers who have studied this subject in Europe and America have found that tubercle bacilli of bovine type are frequently met with in the tubercular

cervical glands of children. We have, therefore, selected these diseased glands for study. Considerable difficulty has been experienced in collecting material and in making our cultures. We are particularly indebted to Dr. Benson of the Cama Hospital for the glands she has sent us. We hope to be able to give an account of this important work in our next annual report.

VIII.

Diarrhœa investigation in Poona.

21. Captain Morison, I.M.S., submitted a report to Government on the result of his inquiry into the causes of diarrhœa in Poona during the rainy season. His investigations suggested the possibility that the water-supply which is derived from Lake Fife and Karakvasla canal was largely responsible for the disease. He pointed out that some 16 villages with eight thousand inhabitants are situated around the lake while in the valleys which drain into the lake sixty-six more villages are found. The bacteriological and chemical analysis of the water in his opinion favoured the view that diarrhœa in Poona during the rains was due to the pollution of the lake.

Owing to the considerable expenditure which must be incurred in improving the water-supply of the city, it seemed desirable that this inquiry should be prolonged and even more convincing evidence than that submitted by Captain Morison should be accumulated during another year.

IX.

The treatment of Leprosy by Williams' Vaccine.

22. The treatment of Leprosy with a vaccine prepared from the streptothrix isolated by Major Beauchamp Williams, I.M.S., was continued throughout the year. Vaccine was supplied to a number of persons in India and other parts of the world. On account of the slow progress of the disease, the recurring attacks of fever with extension of the leprosy lesions and the periods of remission and temporary improvement even in untreated cases, it is difficult to arrive at any conclusion as to the usefulness of the vaccine as a method of treatment. Generally speaking it may be said that some cases appear to improve under treatment, some remain stationery, while others show no improvement at all.

The following extract from a report by Major Tyrrell, I.M.S., from Nowgong, is an example of a number of other similar reports. He states that "in all 16 cases were treated during the year..... From seven of the cases nothing can be learnt as they only received from one to six doses. From the remaining nine some information can be gathered and I enclose a short note on each case. My conclusions are that of these nine cases, seven improved by treatment, the remaining two showing very slight, if any, improvement. Not one of the nine was worse at the end of the treatment than at the beginning although one had been under treatment for 10 months, one for 8, one for 7, two for 4, two for 3, and two for 2."

(c) *Assistance and Advice rendered.*

Examination of mosquito larvæ for the Bombay Municipality.

23. The following statement shows the number of samples of mosquito larvæ received from the Bombay Municipality for identification :—

Species.	No.	Total.
Stegomyia fasciata	728	
Stegomyia scutellaris	59	
Scutomyia sugens	26	
Desvoidea obturbans	87	
Culex co: color	613	
Culex fatigans	1,514	
Taeniorhynchus perturbans	27	
		3,054

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Species.	No.	
Anopheles stephensi	954	
„ rossi	1,579	
„ jamesi	4	
„ culicifacies	2	
„ barbirostris	41	
„ listoni	1	
Mansonia nigra	24	
		2,604

Scientific papers read by the staff of the laboratory.

24. The staff of the laboratory prepared the following papers for the All-India Sanitary Conference held in Lucknow :—

"A preliminary enquiry into the Prevalence of Tuberculosis amongst Bombay Cattle" by Major T. H. Gloster, M.B., D.P.H., I.M.S.

"The Distribution of Guineaworm in India" by Dr. D. A. Turkhud, M.B., C.M. (Edin.).

"Guineaworm Disease in an Indian Village" by Major W. Glen Liston, C.I.E., M.D., D.P.H., I.M.S., and Dr. D. A. Turkhud, M.B. C.M. (Edin.).

"Further investigations into the position of Plague Infection in the villages of Poona District during the Off-season" by Captain J. C. G. Kunhardt, I.M.S.

"Plague Prophylactic Measures during the Off-season" by Captain J. C. G. Kunhardt.

"Hydrocyanic acid as a disinfectant for plague houses" by Major Liston and Captains Stevenson and Taylor.

25. Major Liston was invited to open the discussion on plague at the Seventeenth International Congress of Medicine held in London from the 6th to 12th August 1913. At the Congress he read two papers on Plague: one contrasting the epidemiological features of bubonic and pneumonic plague and the other on the immunity of *mus rattus* to plague infection.

(*d*) *Educational.*

26. Arrangements were made to give a series of popular lectures and demonstrations to High School Teachers and students on the cause and prevention of disease. Three hundred and twenty students in all attended these lectures and demonstrations.

27. Special demonstrations were arranged at the request of Dr. Turner and his Assistant Dr. Shroff for the Sanitary Inspectors' class and at the request of Dr. Surveyor for the fifth-year students in medicine attending the Grant Medical College.

28. Lantern slides illustrating matters concerning the public health have been supplied to a number of District Sanitary Associations in the Bombay Presidency.

29. An exhibit was prepared and despatched to the International Exhibition at Ghent, illustrating the work which has been carried out at the laboratory in the elucidation of problems relating to the cause, prevention and treatment of plague.

30. Some progress was made in the proposals and plans for founding a School of Tropical Medicine at the laboratory.

31. The continued progress of the laboratory has only been made possible by the loyal support and the willing help I have at all times received from the whole staff of the laboratory.

W. GLEN LISTON, M.D., D.P.H.,
Major, I. M. S.,
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